Evaluation of immune status against *Babesia Bigemina* in cattle after treatment with imidocarb dipropionate and diminazene aceturate.

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Abstract

The aim of this study was to evaluate the hemogram and antibody titre after treatment of *babesia bigemina* infected animal with imidocarb dipropionate and diminazene aceturate. 30 male, native breed cattle (12-14 month age) were used in this study. Ten animals were used as control healthy group (free of *babesia* infection) and the other 20 animals were used as infected group as proven by using blood film examination. Results showed significant decrease of RBCs count, Hb concentration and PCV values in infected animals before treatment. Total leukocytes, neutrophils, and monocytes counts showed significant increase, while lymphocyte count showed significant decrease. Treatment with either imidocarb dipropionate or diminazene aceturate improved hemogram changes depending on periods from the beginning of treatment. Serum antibody titre was determined by indirect fluorescent antibody test (IFAT). It increased after one week of treatment. The antibody titre was high after 2 weeks of treatment with imidocarb more than after diminazene treatment. The results of this study demonstrate that the possibility of reinfection with *babesia bigemina* in cattle is lower after imidocarb dipropionate treatment due to the high level of protective antibodies than after treatment with diminazene aceturate. .

Key words: *babesia bigemina*, antiparasitic drugs, IFAT

Introduction

Babesia species are hemoprotozoan parasites of animals which are transmitted by ticks. *Babesia* parasites infect a wide variety of wild and domestic animals, and enormous economic losses due to babesiosis are reported throughout the world ***(Kuttler, 1985)***. Anemia is the major symptoms and cause of mortality in infected animals. In general, *Babesia* parasites invade erythrocytes of infected animals, resulting in the destruction of the parasitized erythrocytes ***(Kawamura, et al. 1987).*** Increase the level of anti-erythrocyte membrane antibodies and erythrocyte-bound IgG in dogs infected with *Babesia gibsoni* have been reported ***(Farwell, et al. 1982),*** indicating the immune destruction of both parasitized and non-parasitized erythrocytes. The treatment with anti-Babesial drugs was effective in clearing the infection, but the maintenance of protective antibodies, making the animals more resistant to reinfection ***(Brandão, et al. 2003).*** Therefore this study was designed to diagnose *Babesia* infection using indirect fluorescent antibody test (IFAT) and monitoring the antibodies titre in serum of infected animals before and after treatment with imidocarb dipropionate and diminazene aceturate.

Material and methods

Animals

30 male, native breed cattle (12-14 month age) were used in this study from a cattle farm in Moushtohur, Kalubyia province. Ten animals were used as control healthy group (free of babesia infection) and the other 20 animals were used as infected group as proven using blood film examination. The infected group was subdivided into 2 subgroups (ten animals in each subgroup). The first subgroup was treated with imidocarb dipropionate (1.2 mg/kg b. wt.) and the second subgroup was treated with diminazene aceturate (3.5 mg/kg b. wt.) respectively.

Drugs

a- Imidocarb dipropionate (Imizol) was obtained from Schering-Plough Animal Health Pharmaceutical Company.

b- *Diminazene aceturate (Berenil)* was obtained from Intervet pharmaceutical company.

c- *Conjugate:* fluorescence labeled (FITC) purified antibody to bovine IgG developed in rabbit was obtained from Sigma Immunochemical Company. The conjugate was dilutes in PBS (1:16) and reacts with bovine IgG of infected bovine serum.

d- *Bovine serum albumin:* it was obtained from Sigma Immunochemical Company and used for antigen preparation for IFAT.

Blood tests

Blood samples were collected before and after treatment by 2 days, one week and 2 weeks on EDTA as anticoagulant for hemogram evaluation which includes; total red blood cell count, hemoglobin concentration, packed cell volume, red cell indices, reticulocyte count, total leukocytic count and differential leukocytic count according to ***Duncan, et al. (1994).***

Indirect fluorescent antibody test (IFAT)

a- *antigen preparation*

Antigen preparation and test procedure was performed according to ***Akinboad and Dipeolu (1984) and Hegazy et al (2003).*** Whole blood with *Babesia Bigemina* from infected calves was drawn into tubes containing EDTA as anticoagulant. The blood was then centrifuged at 300 rpm for 30 minutes and the plasma and buffy coat were removed. The infected red cells were washed three times in phosphate buffer saline (pH 7.2) by centrifugation for 10 minutes at 3000 rpm. The washed red cells were resuspended in PBS containing 1.75% bovine serum albumin in ratio of 2:3 respectively. Thick blood films were prepared from red cell suspension then air dried. Each slide was wrapped by masking tape and stored at – 70 °C until used.

b- *IFAT procedure:*

1- The antigen slides were removed from -70 °C and placed in a desiccator jar for 30 minutes at room temperature.

2- The masking tape was removed and the slides were fixed in cold acetone for 5 minutes and then air dried.
3- Wells were made on the surface of each slide by using nail polish.
4- Serum samples from control positive and control negative were applied to wells on the surface of the slide (5µl/well) at a dilution of 1:40 in PBS.
5- Then the slides were incubated in humid chamber at 37 °C for 30 minutes.
6- Washing twice with PBS and once with distilled water (for five minutes) was performed.
7- Diluted conjugate was applied to each well (5 µl/well), then the slides were incubated for 30 minutes in humid chamber at 37°C.
8- Washing by PBS was performed as the previous step, and then the slides were mounted with buffered glycerol and examined under fluorescent microscope.
9- The Appearance of apple green fluorescent parasites was considered as positive reaction for Babesia.
10- To study the IFA titre of the resulted positive sera, a double fold dilution beginning from 1:40 – 1:640 were performed and proceeded as described above.

Statistical analysis

Data are expressed as the mean ± SE. differences between means were analyzed by T test. A *P* value of less than 0.05 was considered significant according to ***Snedecor and Cochran (1994).***

Results

Blood smear examination

Examination of Giemsa stained blood smear from infected animals reveled presence of different developmental forms of *Babesia bigemina*. Double bear form was the most common form. Other forms also appeared such as single bear form, oval form and round form as shown in figure (1).

Indirect fluorescent antibody test (IFAT)

Negative IFAT was observed in control healthy animals, infected animals before treatment and two days after treatment as shown in figure (1-B). Positive reaction was found in the treated groups after 1 and 2 weeks of treatment with imidocarb dipropionate (Imizol) and diminazene aceturare (Berenil); and was indicated by the appearance of apple green fluorescent parasites as shown in figure (1-C)

The antibody titre for *Babesia* obtained from the double fold serial dilution for sera was zero in control healthy animals, infected animals before treatment, and two days after treatment with Imizol and Berenil. After that the antibody titre increased after one week of treatment with Imizol reached 64 and 432 after two weeks; while treatment with Berenil showed antibody titre 52 and 352 after one and two weeks of treatment respectively as shown in table (3).

Hemogram:

Significant decrease ($p<0.001$) of RBCs count, hemoglobin concentration and PCV value was observed in infected animals before treatment as shown in table (1). Treatment with Imizol and Berenil significantly increase RBCs, Hb and PCV compared to the values of untreated infected animals (table 1, 2)

Total leukocyte count showed significant increase ($p<0.05$) in infected untreated animals compared to control healthy ones. Differential count showed significant increase in neutrphils and monocytes, and non significant increase in eosinophils and basophils; while lymphocytes showed significant decrease as shown in table (1). Treatment with Imizol and Berenil showed decreased total leukocyte, neutrophils, and monocytes counts after treatment and become significant after one and two weeks of treatment; while lymphocytes showed significant increase after one and two weeks from treatment compared to the values before treatment (table 1,2).

Table (1): Hemogram of infected animals before and after treatment with imidocarb dipropionate (Imizol). (Mean ±SE)

	Control healthy.	Control infected	Two days of treatment.	One week of treatment.	Two weeks of treatment.
RBCS ($x10^6/\mu L$)	7.39 ± 0.29	4.93 ± 0.13***	5.60 ± 0.30*	6.92 ± 0.28***	7.21 ± 0.18***
Hb (gm%)	10.98 ± 0.36	7.86 ± 0.11***	8.34 ± 0.07**	9.94 ± 0.13***	10.44 ± 0.07***
PCV (%)	33.90 ± 0.65	25.85 ± 0.41***	29.10 ± 0.76***	31.6 ± 0.70***	33.20 ± 0.62***
T.L.C. ($x10^3/\mu L$	7.60 ± 0.41	8.64 ± 0.16*	8.38 ± 0.22	8.13 ± 0.15*	7.92 ± 0.23*
Neutrophils	2.55 ± 0.25	3.93 ± 0.21***	3.43 ± 0.28	3.09 ± 0.27*	2.82 ± 0.25**
Eosinophils	0.08 ± 0.03	0.15 ± 0.02	0.11 ± 0.02	0.10 ± 0.02	0.09 ± 0.03
Basophils	0.03 ± 0.01	0.07 ± 0.01	0.05 ± 0.01	0.04 ± 0.02	0.03 ± 0.01
Monocytes	0.21 ± 0.02	0.52 ± 0.05***	0.483 ± 0.062	0.34 ± 0.04*	0.26 ± 0.06**
Lymphocytes	4.82± 0.14	3.98 ± 0.10***	4.29 ± 0.29	4.52 ± 0.23*	4.67 ± 0.18**

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Table (2): Hemogram of infected animals before and after treatment with diminazene aceturate (Berenil) (Mean ±SE)

	Control healthy.	Control infected	Two days of treatment.	One week of treatment.	Two weeks of treatment.
RBCS ($x10^6/\mu L$)	7.39 ± 0.29	4.93 ± 0.13***	5.81 ± 0.23 **	7.11 ± 0.23***	7.32 ± 0.19***
Hb (gm%)	10.98 ± 0.36	7.86 ± 0.11***	8.98 ± 0.07***	10.16 ± 0.08***	10.52 ± 0.12***
PCV (%)	33.90 ± 0.65	25.85 ± 0.41***	30.3 ± 0.63***	32.3 ± 0.78***	33.4 ± 0.60***
T.L.C. ($x10^3/\mu L$	7.60 ± 0.41	8.64 ± 0.16*	8.51 ± 0.252	8.11± 0.25	7.74 ± 0.33 *
Neutrophils	2.55 ± 0.25	3.93 ± 0.21***	3.641± 0.188	3.095 ± 0.320 *	2.65 ± 0.26***
Eosinophils	0.08 ± 0.03	0.15 ± 0.02	0.14 ± 0.03	0.10 ± 0.01	0.08 ± 0.02
Basophils	0.03 ± 0.01	0.07 ± 0.01	0.06 ± 0.02	0.04 ± 0.01	0.03 ± 0.01
Monocytes	0.21 ± 0.02	0.52 ± 0.05***	0.47 ± 0.03	0.30 ± 0.04**	0.24 ± 0.03***
Lymphocytes	4.82± 0.14	3.98 ± 0.10***	4.18 ± 0.32	4.58 ± 0.21*	4.74 ± 0.21**

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Table (3): Serum antibodies titre for *B. bigemina* (Mean ± SE)

	Imizol treated group			Berenil treated group		
	2 dayes	**1 week**	**2 weeks**	**2 days**	**1 week**	**2 weeks**
Antibodies titre[H]	0	64 ± 6.53***	432 ± 58.66***	0	52 ± 6.11***	352 ± 32***

The IFA titre was expressed as the reciprocal of the highest serum dilution at which parasite fluorescence was observed.

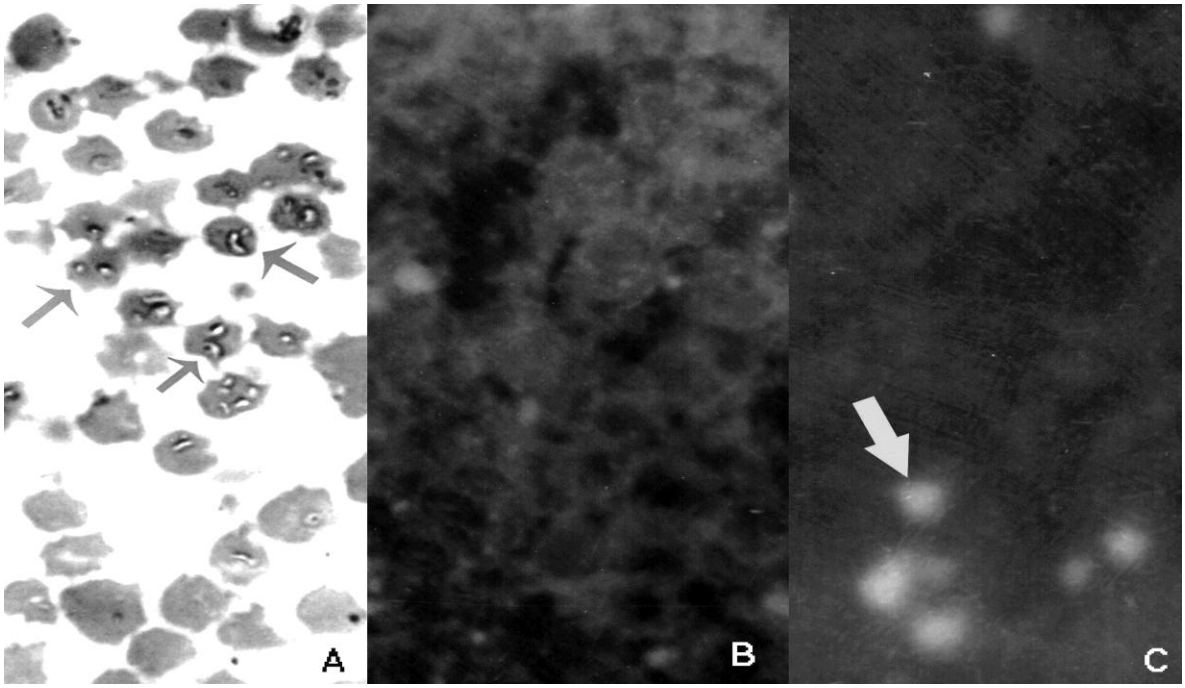

Figure (1) A- infected red cells with *Babesia Bigemina* (Giemsa's stain). B- Negative IFAT with control healthy serum. C – Positive IFAT with infected serum

Discussion

Babesiosis is an enzootic disease in tropical and subtropical countries leading to significant loss in meat and milk production. Identification of *Bebesia* protozoans in the blood smear represents a true evidence of infection. In Carrier animals and chronic stages of the infection, detection of the parasite is difficult, therefore serological methods is used to detect specific antibody of *babesia* species rather than the *babesia* organism. ***(Ashmawy et al., 1998).*** In the present study blood smear from infected animals showed different morphological forms of *babesia bigemina* such as double and single bear forms, round and oval forms. These morphological forms represent the developmental stages of the parasite as reported by ***Bhikane et al., (2001***) ***and Homer et al., (2000)***. Babesia invaded erythrocytes of the infected animals resulting in their destruction, which was reflected by hemoglobinuria and a significant decrease in red blood cell count, hemoglobin concentration and packed cell values. These results agree with ***Rosignoli et al., (2000) and Yeruham, et al., (1998).*** Treatment with imidocarb dipropionate and diminazene aceturate decreased the parasites from the blood, which improved RBCs count, Hb and PCV gradually after 2 days, one and two weeks. These results agree with ***Mamdouh (1998).*** Leukogram in infected non treated animals showed significant increase in total leukocyte, neutrophils, and monocytes counts, while lymphocytes showed significant decrease. Eosinophil and basophils showed no significant increase. These results agree with ***Camacho et al., (2002).*** This leukogram indicates stress condition from *babesia bigemina* infection as explained by ***Coles (1986).*** Treatment with imidocarb dipropionate and diminazene aceturate improved leukogram as indicated by increased lymphocyte and gradual decrease of total leukocyte count to normal values.

Protective immunity against reinfection with *babesia bigemina* is indicated by antibody titre after infection or treatment by antibabesial drugs as described by ***Igarashi, et al., (1999*)**. Antibodies for *babesia bigemina* appear in serum after one week of infection and reach maximum after 4 weeks as reported by ***Kolabskii, et al., (1973); Lewis et al., (1980) and Hegazy, et al., (2003).*** In this work the antibody titre to *Babesia bigemina* was zero in infected animals before treatment and 2 days after treatment with

either imidocarb dipropionate or diminazene aceturate. Antibody titre increased gradually after one and two weeks of treatment. Imidocarb dipropionate treatment gives a high antibody titre more than diminazene aceturate after 2 weeks of treatment as indicated by IFAT. Therefore the possibility of reinfection with *babesia bigemina* in cattle is lower after imidocarb dipropionate treatment due to the high level of protective antibodies than after treatment with diminazene aceturate.

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